

STEERING COMMITTEE MEETING NOTES

Date: May 27, 2003

Type of Meeting: Conference Call

Attendees: Adam Arkin, Terry Hazen, Jay Keasling, Vince Martin, Anup Singh, Judy Wall, Martin Keller, David Stahl, Joe Zhou, Melissa Ramos and Nancy Slater.

Current Progress:

1. Planning to create a critical path for the project was discussed. This critical path includes the oxygen stress experiments for *Disulfovibrio*. **ACTION: Nancy will contact Jim Fredrickson (PNNL) regarding the oxygen stress data for *Shewanella*. Jim offered to give our GTL project team members access to his discussion boards (email Jim to request a password Jim.Fredrickson@pnl.gov).**
2. Judy is needs a method for measuring dissolved oxygen in sulfide containing bacteria. Judy is having problems with her chemostat setup in her lab. Batch cultures may be necessary if the chemostat is not available. Everyone agrees that pH metering is necessary in these experiments. Joe has batch culture data that are consistent with chemostat data.
3. **ACTION: Judy will research literature to see if she can find information regarding oxygen stress experiments on sulfur reducing bacteria.**
4. **ACTION: Joe needs to post his protocols in Biofiles.**
5. Vince Martin measured phospholipids and fatty acids in the presence of oxygen – the cells were in the Log stage of development for this experiment. Each group needs to grow the bacteria using the same conditions (temperature, pH, growth rate).
6. **ACTION: Metabolomics & Proteomics groups need cells from Terry. Martin will email his request, and Joe needs 10^{10} cells. Terry will give descriptions of his media preparation by the end of this week.**
7. **WARNING:** Judy warned against filtering LS media when it is hot instead of warm, and she will change the protocol in Biofiles. Judy also suggests that you look at the cultures from time to time to make sure that there is no contamination.

Future Work:

8. Terry is looking at activity indices using synchrotron FTIR, TEM and SEM.
9. Jay is using an ICP method that is working well. He is working on heat shock experiments.
10. Martin is performing whole proteome experiments to see how many proteins he can see; he will compare his data to Jay's data.
11. Joe will have the *Disulfovibrio* microarrays complete by the end of June.
12. **ACTION: Joe will send his protocol for storage techniques to Vince Martin.**
13. David Stahl posted the sequence alignment and phylogenetic tree to Biofiles. He noted that the PT2 species may be a candidate for future studies.
14. **ACTION: David needs sediment samples collected at deeper locations. Terry will talk with Dave Watson (sampling crew) to see if he can get some fresh sample.**

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15. There was a discussion regarding co-culturing *Disulfovibrio* and a methanogen to measure hydrogen, methane, etc. in the absence of sulfate; measurements could be made to compare the response to stressed conditions versus non-stressed conditions using PCR based array methods.
16. **ACTION: Adam will review Anup's heat shock experimental data.**
17. Vince Martin would like to obtain the G20 strain from Bill Metcalf for genetic manipulation.
18. **Vince Martin would like to coordinate the first oxygen stress experiment. He will coordinate with Terry's group to set up the cell culture conditions, biomass harvesting and distribution. Vince will send out a detailed protocol of the experiment before he begins the experiment.**
19. Vince is thinking about gene knockouts, and he is thinking about starting with two-component regulators. Alyssa is a graduate student in Jay's lab that is ideas for her PhD thesis and may be interested in helping with developing the knockouts. Judy would like to start targeting mutagenesis. Adam suggested using barcodes (from the yeast project).
20. **ACTION: Nancy needs to discuss financial reporting with Joe at ORNL.**

End of Report